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SECTIONING REFRACTORY WOODS FOR ANATOMICAL STUDIES.(U)
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SECTIONING REFRACTORY WOODS FOR ANATOMICAL STUDIES

By

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Abstract

Describes a new technique for softening wood, using a 4 percent solution of ethylenediamine; a shortcut method for removing silica and crystalline materials with hydrofluoric acid; a method for rapidly neutralizing wood blocks which have been treated with hydrofluoric acid; and a method for maintaining the flatness of sections through staining, dehydration, and clearing. Methods described are applicable to wood with a specific gravity in excess of 0.75.

Introduction

The difficulties encountered in the preparation of microtome sections of wood are fully appreciated by wood anatomists, particularly when the wood being sectioned is in a higher density class and contains silica, as is the case of many species of the Chrysobalanaceae, Lecythidaceae, and Sapotaceae.

1/ Pioneering Research Unit

Described here are techniques which were developed during an anatomical study of the Sapotaceae and differ from all previously established guidelines. Highly satisfactory sections and slides can be produced in a remarkably short period of time and with a minimum of frustration. It is believed that these procedures will be of benefit to the experienced but especially so to the neophytes.

The author would be the first to admit that the procedures described are not the "last word" and that improvements will certainly occur with respect to the time-temperature schedules. However, the fact that it was possible to prepare sections of 200 species of Sapotaceae in a month should lend courage to those who have avoided the study of dense woods simply because they were judged "too tough" or impossible.

Reagents and Equipment

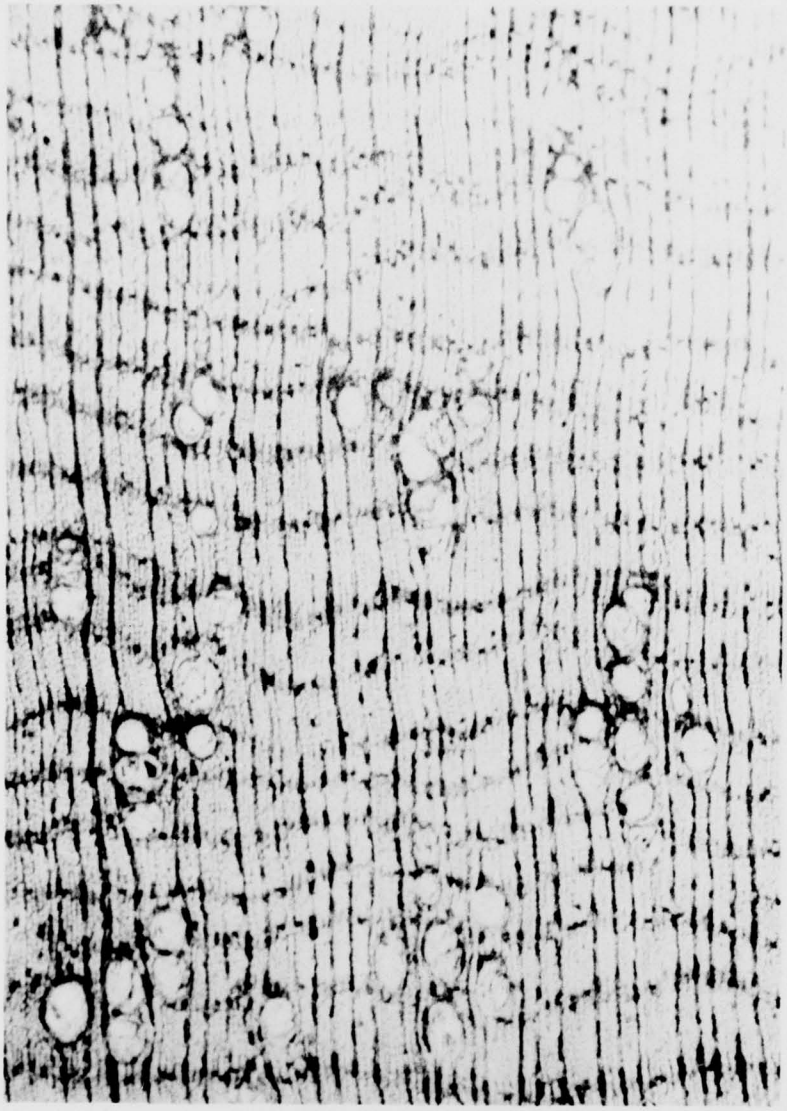
The reagent used for softening the wood is ethylenediamine (hereafter referred to as ETD) and is generally available in concentrations of 98 to 99 percent. The full-strength solution is an irritant to the skin and the fumes are very irritating to the eyes and nose, so handling should be in keeping with safe laboratory practice. In this procedure, a 4 percent solution of ETD in distilled water is utilized. Wood blocks which have been treated in the 4 percent solution can be handled with the bare hands, the solution does not fume, and microtome knife edges are not affected.

ETD is a swelling agent which increases the cell wall volume slightly beyond that obtainable by simply saturating in water. In effect, this reduces the density of the cell wall and thus makes sectioning of dense woods practicable. The swelling induced by the ETD is practically eliminated during subsequent procedures. This solution has no effect on silica, crystals, and other cell inclusions.

Hydrofluoric acid (HF) is used in this procedure to remove silica and crystalline materials from the wood blocks which furnish transverse sections. Without this treatment it is impossible to prepare high-quality transverse sections of dense woods. Commercial HF is usually available in a solution strength of 48 percent. In this procedure the solution strength is reduced by one-half by mixing equal volumes of commercial HF and distilled water.

As with other strong reagents, extreme care must be exercised in the handling of HF solutions.

The only equipment required is a vacuum-producing system and a heat source such as an electric hot plate. The vacuum system used in these procedures consisted of a dry-seal vacuum dessicator with a sleeve-valve



Example of success--a wood that had never before been successfully sectioned was cut quickly by this new method. Myrtiluma eugeniaefolia (Pierre) Baillon is a wood from Surinam, with a specific gravity of 1.20. Fibers are extremely thick walled, sclerotic tyloses, sclerotic parenchyma and wood rays. The ray cells contain silica.

coupled to an aspirator. The sleeve-valve permits the vacuum to be placed on "hold" for varying periods of time.

The author uses a Reichert sledge-type microtome for sectioning refractory wood. For sectioning with a cutting angle of 15° a knife setting of 15 (on the instrument) was most satisfactory. The cutting angle is the angle between the knife edge and the direction of knife travel. It is extremely important that the knife be firmly secured in its holder and that all clamping screws and clamping levers are tight. All sections were cut at a setting of 22 microns.

Sectioning Blocks

Prepare the sectioning blocks carefully to avoid disappointment at the time of sectioning. The wood collection specimen from which the blocks are to be cut should be examined very carefully, paying particular attention to the transverse surfaces. If checking is present in the specimen, determine how far along the grain these checks extend. If both transverse surfaces appear to be excessively checked, it may be necessary to take the sectioning blocks from the central portion of the specimen. Fortunately, the great majority of collection specimens will have one transverse surface that is free of checking, or the checks may be sufficiently spaced so that blocks of satisfactory size may be obtained between the checks. In high-density woods, checking may be present but not readily detectable with a hand lens. In these instances the checking may not be detected until sectioning has been initiated.

When ample material is available, two blocks are prepared, one of which will provide transverse and tangential sections and is taken from the "bark side" of the wood collection specimen. The other will provide radial sections and is taken from the "pith side" or the heartwood in order to show the mature development of silica, crystals, and other inclusions. The blocks which will provide radial sections are never treated in HF in order that the cell inclusions remain in their natural condition.

It is the general practice to aline the sectioning block from which transverse sections will be cut so that the wood rays will parallel the direction of knife travel. In dense woods this is not practical because the wood rays are a zone of weakness, and artificial checking will be produced by the shearing action of the microtome knife. In dense woods it is necessary to aline the sectioning block so that the wood rays are at an angle with respect to knife travel. The best results have been obtained when the rays are 45° to 90° with respect to knife travel. Because of this situation, the sectioning block which will provide transverse sections should be cut so that the longest dimension will be approximately in the radial direction and the shortest dimension will be in the tangential direction.

The ideal sectioning block will then measure approximately 2 centimeters long radially, about 1 centimeter in width tangentially, and approximately 2 centimeters in length along the grain. The 2-centimeter dimensions may appear to be excessive but will provide fine sections which, after trimming, will fit comfortably under a cover glass. The blocks for radial sections should be prepared similarly in order to provide sections which will measure approximately 1 centimeter in radial width and 16-18 millimeters in length along the grain after trimming. The three planes of view are mounted under a cover glass which measures 22 by 40 millimeters.

When preparing the wood blocks for sectioning, one should always bear in mind the alinement of the tissues with respect to the plane of sectioning. The transverse surface must be at a right angle to the grain direction and parallel to the wood rays as viewed from the radial face of the block. The radial surface must be parallel to the wood rays and with the grain direction. The tangential surface must be parallel with the grain and at a right angle to the wood rays. This is a very important consideration because in dense woods one will have very little leeway in producing fine sections if excessive trimming or alinement is required after treatment.

Specific Gravity

It is quite obvious that a light wood or one moderately light in weight will be easier to section than a hard, dense wood and especially a wood that will sink in water. For this reason it is important to know the specific gravity of the wood to be sectioned. As applied to wood, the specific gravity is defined as the ratio of the weight of the sample to the weight of a volume of water equal to the volume of the sample at a specified moisture content.

In this paper, the specific gravity values cited are based on specimens from the Forest Products Laboratory collection with an average moisture content of 6 to 7 percent. The samples were weighed to the nearest 0.1 gram and the volume determined by water displacement to the nearest 0.1 milliliter. The weight in air divided by the volume gives the specific gravity of the specimen.

For wood collection specimens of uniform cross section, another simple method may be used. This is accomplished by lowering the wood specimen slowly into a beaker or cylinder of water but always maintaining the specimen in a vertical position. When the specimen no longer sinks in water, remove the specimen and mark the position of the water line. Measure the specimen iength and the total submerged length. The submerged length divided by the total specimen length will give the approximate specific gravity for the wood specimen.

Specimens that sink in water will obviously have a specific gravity value of greater than 1.00 and floaters will have a value of less than 1.00.

When the specimens are of uniform dimension, the specific gravity can also be determined by weighing the specimen and determining the volume by actual measurement. The weight divided by the volume will give the specific gravity.

In a recent anatomical study of the Sapotaceae, more than 1,600 specimens with specific gravities from 0.38 to 1.33 were sectioned; beyond a specific gravity of 0.75-0.80, more softening was required than is obtainable by prolonged boiling in water. Below a specific gravity of 0.55 it is only necessary to saturate the blocks in water; this process may be hastened by the application of a vacuum. Above 0.55 it is generally desirable to heat the saturated blocks in water for 1-2 hours to reduce the tendency of the sections to roll or curl as they are removed from the knife.

Treatment with Ethylenediamine (ETD)

The previously prepared dry sectioning blocks are placed in a 4 percent solution of ETD in small beakers (250 ml). Hold-down disks of brass or bronze about 5 millimeters thick, and of a smaller diameter than the beaker, are used to keep the wood specimens submerged in the solution. The hold-down should have a central rod which projects above the top of the beaker to permit easy removal. Sinkers obviously do not require the hold-down weights. Place the beaker in a vacuum-type desiccator, evacuate for approximately one-half hour, and release vacuum. Repeat this procedure about three times. Then apply a final vacuum and place on "hold" for a period of 12 to 16 hours. This treatment time is applicable to floaters or those woods with a specific gravity of less than 1.00.

For sectioning blocks that are sinkers, or have a specific gravity of 1.00 plus, repeat the above procedure but hold the final vacuum for 24 to 32 hours.

After this treatment, pour off the discolored ETD solution and replace with a fresh solution. Smooth and aline the radial and tangential surfaces with a reasonably sharp knife. At this point wood may be exposed which has not been treated or saturated with ETD. If so, the untreated area will be most evident on radial surfaces because the permeability of wood is lowest in this direction.

At this point an attempt should be made to smooth and aline the transverse surface. However, this may or may not be successful except for those woods which are lower than 1.00 in specific gravity. After smoothing and alining, return to vacuum for about 1 hour.

Following the vacuum treatment, wood with a specific gravity of less than 1.00 is heated slowly, bringing to a temperature of 70°-75° C., which should take about one-half hour. After this heating, radial and tangential sections may be cut directly from the ETD treated blocks. It is not necessary to rinse or wash the blocks in water but simply remove the excess ETD with an absorbent paper towel. At this point it is generally possible to smooth and aline the transverse surface to the desired condition.

The block that will be used for the production of transverse sections is now returned to the ETD solution, again slowly heated to a temperature of 70°-75° C., and held at this temperature range for about 15 minutes. At the end of this final heating period, remove the blocks from the hot solution with tongs, and blot with a paper towel, allowing the block to surface dry for about 1 minute. Then place immediately in a 50-50 solution of HF and water for a period of about 16 hours (a longer period will have no adverse effects).

After the HF treatment, remove the blocks with tongs and place in a saturated solution of sodium bicarbonate. Apply vacuum for 4 hours or overnight, if convenient. Because of the corrosive nature of HF and the possibility that pockets of HF in the wood have not been neutralized, it is imperative that the "lubricant" or sectioning solution also be a saturated solution of sodium bicarbonate.

Care of Sections

Sections cut from dense woods exhibit a decided tendency to curl or, if the wood has not been adequately softened, to roll up tightly when placed in a dish of water. A small amount of curling or rolling of the sections at the edge of entry of the microtome knife and at the exit edge is to be expected, and indicates that the block has been softened nearly to perfection. The curled or rolled portions are easily trimmed from the section to leave a very ample transverse section for study. Several methods of preventing the rolling of sections may be found in the literature but generally these have proven inadequate with respect to woods of high density.

The method used by the author consists of placing the sections between glass slides, clamping at the ends with No. 2 paper binder clips (which are 2 cm wide and have an effective opening of approximately 1 cm), and heating the clipped bundles in distilled water for several hours.

In practice, the sections (usually four) from a given plane are placed on the slide as they are sectioned and immediately covered with another slide. Thus when a species is completely sectioned, one will have a bundle of six slides to which is added a paper label bearing the wood

specimen number or other pertinent data applied with waterproof ink; this in turn is covered with another slide for additional protection. It has been the practice of the author to heat the slide bundles for several hours in the afternoon, turn off the hot plate but allow the beaker of water to remain on the plate overnight. The next day, or at some convenient time, the slides may be separated, the sections trimmed, and staining and slide preparation may be initiated. Sections that may adhere to the slide, as those near the clips, can be readily freed by sliding the sharp edge of a razor blade under the section.

Of the more than 1,600 specimens of Sapotaceae that were so treated there was no instance of rolling when the sections passed through the staining, dehydration, and clearing process. Occasionally the transverse sections may assume a rather loose or "open" roll when placed in alcohol but these flatten out in the toluene used for clearing. The author used safranin exclusively for staining purposes with excellent results.

Supplementary Notes

1. If several species are being processed in the same container, each block must be identified by a number or letter. Because many wood collection specimens may have five-digit collection numbers and because space on the sectioning block is usually limited, the use of a single digit code number is the wisest procedure. This code number is generally applied to all surfaces which will not be sectioned. If the ink is applied to a surface intended for sectioning it may penetrate deeply enough to be visible on the cut sections. The waterproof ink used in many of the common fiber-point pens will readily survive the treatment with ETD and HF.

2. If processing several species in the same container, it is imperative to keep the specific gravity variation between species within rather close limits. A variation of 0.05 apparently is tolerable, but even with this slight variability one may encounter considerable differences in permeability and thus differences in ease of sectioning.

3. Blocks do not necessarily have to become saturated to the point that they sink in the ETD solution. This may require an inordinately long time and very likely the block would become useless for sectioning. For the treatment periods recommended, the ETD solution will penetrate several millimeters into the most difficult woods; this penetration is usually adequate.

4. The wood is still too hard if the knife skips on the block during sectioning or the sections show zones of uneven thickness. If this happens, the block should be returned to the ETD solution and heated for 20 to 30 minutes at a temperature not exceeding 75° C. Before

reheating, check all clamps for tightness and the condition of the sectioning knife. A slightly dull knife may skip whereas a precision-sharp knife will produce satisfactory sections. If the skipping is not pronounced, it may frequently be eliminated by reducing the tangential width of the blocks 1 or 2 millimeters.

5. If sections crumble during sectioning or have a dull appearance and show no tendency to roll, the block has been heated to a higher than suggested temperature or the block was heated too long. Occasionally this may be a superficial condition, and continued sectioning may produce satisfactory sections.

6. The author routinely uses the HF method to treat all blocks from which transverse sections are to be cut. The net result is the production of high-quality transverse sections and a prolonged life of the knife edge.

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